Restriction of HIV-1 Replication in Primary Macrophages by IL-12 and IL-18 through the Upregulation of SAMHD1

Eduardo Pauls, Esther Jimenez, Alba Ruiz, Marc Permanyer, Ester Ballana, Helena Costa, Rute Nascimento, R. Michael Parkhouse, Ruth Peña, Eva Riveiro-Muñoz, Miguel A. Martinez, Bonaventura Clotet, José A. Esté and Margarida Bofill

*J Immunol* published online 22 March 2013
http://www.jimmunol.org/content/early/2013/03/22/jimmunol.1203226

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/03/22/jimmunol.1203226.DC1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Restriction of HIV-1 Replication in Primary Macrophages by IL-12 and IL-18 through the Upregulation of SAMHD1

Eduardo Pauls,*,1 Esther Jimenez,*,1 Alba Ruiz,*, Marc Permanyer,*, Ester Ballana,*, Helena Costa,† Rute Nascimiento,† R. Michael Parkhouse,‡ Ruth Peña,*, Eva Riveiro-Muñoz,*, Miguel A. Martínez,*, Bonaventura Clotet,*, José A. Esté,† and Margarida Bofill*†‡

Monocyte-derived macrophages (MDM) can polarize into different subsets depending on the environment and the activation signal to which they are submitted. Differentiation into macrophages allows HIV-1 strains to infect cells of the monocytic lineage. In this study, we show that culture of monocytes with a combination of IL-12 and IL-18 led to macrophage differentiation that was resistant to HIV-1 infection. In contrast, M-CSF–derived MDM were readily infected by HIV-1. When monocytes were differentiated in the presence of M-CSF and then further treated with IL-12/IL-18, cells became resistant to infection. The restriction on HIV-1 replication was not dependent on virus entry or coreceptor expression, as vesicular stomatitis virus–pseudotyped HIV-1 replication was also blocked by IL-12/IL-18. The HIV-1 restriction factor sterile α motif and HD domain–containing protein-1 (SAMHD1) was significantly overexpressed in IL-12/IL-18 MDM compared with M-CSF MDM, and degradation of SAMHD1 by RNA interference or viral-like particles carrying the lentiviral protein Vpx restored HIV-1 infectivity of IL-12/IL-18 MDM. SAMHD1 overexpression induced by IL-12/IL-18 was not dependent on IFN-γ. Thus, we conclude that IL-12 and IL-18 may contribute to the response against HIV-1 infection through the induction of restriction factors such as SAMHD1. The Journal of Immunology, 2013, 190: 000–000.

Monocytes replenish resident macrophages under normal conditions but also move to sites of infection in tissues and differentiate into macrophages in response to immune stimulation. Classically, monocytes may become type M1 macrophages when stimulated through TLRs, CD14, or GM-CSF or become M2 macrophages when stimulated with IL-4 and IL-13 or M-CSF (1–3). However, macrophages have a high degree of plasticity and are profoundly affected by tissue-specific immune-modulating cytokines. Macrophages efficiently respond to environmental signals that change their phenotype and physiology. There is an increasing perception of macrophages as plastic cells that may evolve into a broad spectrum of cell populations depending on the surrounding cytokine environment (4). Indeed, the stimulation of monocytes with a combination of IL-12 and IL-18 (IL-12/IL-18), but not separately, triggers the survival and differentiation of monocytes to macrophages through an inflammatory pathway different from the classical M1 and M2 pathways (5, 6). IL-12/IL-18 also induce the release of a unique pattern of cytokines (IL-8, CCL22, CXCL-9, and CXCL10) that is different from the pattern given by other polarizing stimuli such as GM-CSF or IFN-γ (1). IL-12 appears to cooperate or act synergistically with IL-18 in the control of intracellular parasite infection of macrophages (7) or bridging innate and adaptive immunity (8–10). Notably, treatment of differentiated macrophages with IL-12/IL-18 but not with M-CSF induces the production of IFN-γ (5, 6, 11).

Monocytes are usually resistant to HIV-1 infection but when differentiated into macrophages with M-CSF they become highly susceptible (12). Macrophages are one of the major reservoirs of the HIV type (HIV-1) infection. Once infected, monocyte-derived macrophages (MDM) survive and produce high amounts of viral particles (13).

The importance of macrophages in HIV-1 infection is also highlighted by the discovery of cellular restriction factors found in myeloid cells that protect target cells against viral infections, including HIV-1. A number of host factors alter the expression of HIV receptors and coreceptors, modulate the attachment and entry of virus particles into cells, or restrict HIV replication inside the cell (14). Among these cellular host factors, the sterile α motif and HD domain–containing protein-1 (SAMHD1) regulates the 2′-deoxyribonucleoside 5′-triphosphate (dNTP) pool in MDM, inhibiting the activity of the HIV-1 reverse transcriptase (15–17). SAMHD1 is counteracted by the lentiviral protein Vpx, which is encoded by some SIV strains and HIV-2, but not by HIV-1. Vpx targets SAMHD1 for proteosomal degradation, leading to increased concentration of dNTP and therefore higher levels of HIV-1 replication and virus production (15–18).

In this study, we show that macrophages differentiated in the presence of IL-12/IL-18 were resistant to HIV-1 infection inde-
pendently of HIV-1 coreceptor expression. Moreover, IL-12/IL-18 blocked HIV-1 replication in already differentiated, HIV-1 susceptible, M-CSF MDM. Expression of SAMHD1 was found upregulated in IL-12/IL-18 MDM and, importantly, the down-regulation of this restriction factor with RNA interference or by treatment with viral-like particles (VLP) carrying SIV Vpx protein restored HIV-1 infectivity of IL-12/IL-18 MDM. The IL-12/IL-18-mediated upregulation of SAMHD1 was not prevented by an anti-IFN-γ neutralizing Ab, suggesting that its effect was independent of IFN-γ production. HIV-1+ individuals showed skewed expression of IL-12/IL-18 (19). Thus, IL-12 and IL-18 may contribute to the immune response against HIV-1 infection.

Materials and Methods
Monocytes isolation and macrophage differentiation
PBMC were obtained and differentiated to macrophages as described before (20, 21). Briefly, PBMC were obtained from blood of healthy donors using a Ficoll-Paque density gradient centrifugation. To obtain the monocyte population, 300 million PBMC were blocked with an anti-CD32 Ab (StemCell Technologies, Vancouver, BC, Canada) and monocytes were separated by a negative selection Ab mixture (StemCell Technologies) supplemented with an anti-CD41 Ab (StemCell Technologies) to eliminate platelets or by positive selection using Miltenyi MACS CD14 microbeads (Miltenyi Biotec, Madrid, Spain). The purity of the monocyte isolates was confirmed by flow cytometry staining with a combination of CD14, CD3, CD19, and CD16 Abs (Dako, Barcelona, Spain), analyzed in a FACSCalibur flow cytometer (BD Biosciences, Oxford, U.K.). Platelets were not detected in cell cultures (data not shown). Monocytes were resuspended in complete culture medium: RPMI 1640 medium (Life Technologies, Waltham, MA) supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Monocytes were differentiated for 5–7 d at 50 × 10^3 cells/well in 96-well plates or 1 × 10^5 cells/well in 24-well plates in the presence of M-CSF (PeproTech, London, U.K.) at 20 U/ml (100 ng/ml) or a combination of IL-12 (100 ng/ml; PeproTech) and IL-18 (100 ng/ml; Bionova, Madrid, Spain). When appropriate, differentiated macrophages were cultured in the presence of IFN-γ (100 ng/ml), GM-CSF (100 ng/ml) and IL-4 (250 ng/ml), or TNF-α (100 ng/ml) (all from PeproTech).

RNA interference
Monocytes were transfected with 50 pmol of the corresponding small interfering RNA (siRNA; siGENOME SMARTpool from Dharmacon/Thermo Scientific, Waltham, MA) using a monocyte Amaxa Nucleofector kit (Lonzia, Basel, Switzerland) as previously described (20, 21). Monocytes were left untreated overnight and then differentiated to macrophages as described above.

Virus production and infections
R5-tropic HIV-1 strain BaL was grown in stimulated PBMC and titrated for its use in M-CSF MDM. The macrophage tropic X4 HIV-1 92UG024 was grown in M-CSF MDM. An envelope-deficient HIV-1 NL4-3 clone encoding IRES-GFP has been previously described (22). Virus was produced by cotransfection of the NL4-3-GFP plasmid with a plasmid expressing the envelope protein of the vesicular stomatitis virus (VSV-G) in 293T cells using Lipofectamine. Three days after transfection, supernatants were harvested, filtered, and stored at −80°C. For the production of SIV VLP, 293T cells were cotransfected using polyethyleneimine (Polysciences, Eppelheim, Germany) with pSIV3 (23) and a VSV-G-expressing plasmid. Three days after transfection supernatants were harvested, filtered, and stored at −80°C.

For infection experiments, macrophages were pretreated for 2 h in the presence of VLP and then infected with the VSV-NL4-3-GFP virus. Three days after transfection, cells were detached using a commercial PBS-EDTA solution, fixed, and analyzed by flow cytometry. MDM were infected with the R5 HIV-1 strain BaL between day 3 and day 7 after stimulation. Three days after infection, 100 μl culture supernatant were replaced by 100 μl fresh medium containing the corresponding stimuli. HIV production was analyzed at 7 or 14 d postinfection by ELISA HIV-p24 Ag detection (Bio-Rad Laboratories, Hercules, CA) in culture supernatants.

Quantitative PCR
DNA was extracted using a DNA extraction kit (Qiagen, West Sussex, U.K.), and proviral DNA quantifications were performed as described (21). For relative mRNA quantification, RNA was extracted using the Qiagen RNeasy Mini Extraction kit, as recommended by the manufacturer, including the DNase I treatment step. Reverse transcriptase was performed using a high-capacity cDNA reverse transcription kit (Life Technologies, Madrid, Spain). mRNA relative levels of SAMHD1 were measured by two-step quantitative RT-PCR and normalized to β-actin mRNA expression using the ΔΔCT method. Primers and DNA probes were purchased from Life Technologies.

Immunoblotting
Treated cells were rinsed in ice-cold PBS and extracts were prepared in lysis buffer, subjected to SDS-PAGE, and transferred to a nitrocellulose membrane. The following Abs were used for immunoblotting: HRP-conjugated secondary Ab (Pierce, Hercules, CA), anti-Hsp90 (Beckton Dickinson, Madrid, Spain), and anti-SAMHD1 (Abcam, Cambridge, U.K.). Protein band densities were quantified using ImageGauge software (UVP, Cambridge, U.K.) after acquiring the images with a ChemiDoc-it imaging system from UVP.

Viability assays
For cell viability, MDM were treated as for acute infection experiments. Measurement of cell cytotoxicity was performed by an MTT-based colorimetric assay as described before (24).

**FIGURE 1.** IL-12/IL-18 MD are resistant to HIV-1 infection. (A) Monocytes were differentiated into macrophages for 7 d in the presence of M-CSF, a combination of IL-12 and IL-18 (IL-12/IL-18), GM-CSF and IL-4, or TNF-α. Macrophages were infected with R5 HIV-1 strain BaL and replication was quantified by ELISA measurement of p24 Ag 7 d postinfection. Mean ± SD of one representative experiment is shown. At least three independent donors were tested. (B) Monocytes were differentiated to macrophages for 7 d with M-CSF or IL-12/IL-18 and then infected with HIV-1 BaL. At days 3 and 7, DNA was extracted and proviral DNA quantified by quantitative PCR. Proximal DNA corresponding to one donor was measured in duplicate and is expressed as fold induction relative to a sample of cells infected in the presence of 1 μg/ml HIV-1 inhibitor AZT, as a measure of viral DNA background. One representative of three independent experiments is shown. (C) Monocytes were differentiated to macrophages for 7 d with M-CSF or IL-12/IL-18 and then infected with HIV-1 BaL or 92UG024 strains. HIV-1 replication was quantified by ELISA measurement of p24 Ag 7 d postinfection. Mean ± SD of one representative of three independent experiments is shown.
MDM did not produce p24 Ag (Fig. 1A), as levels were comparable to supernatant (Fig. 1A, filled bars). Conversely, IL-12/IL-18 treatment triggered this HIV-1 restriction, M-CSF–differentiated macrophages were treated for the indicated days with IL-12/IL-18, washed with PBS, and infected for 7 d with HIV-1 BaL. Percentage of p24 production was calculated compared with a control of M-CSF MDM infected for 7 d in the presence of M-CSF. Bars represent mean ± SD of three independent experiments.

**FIGURE 2.** IL-12/IL-18 induces HIV-1 restriction in MDM previously differentiated with M-CSF. (A) Monocytes were differentiated to macrophages for 7 d with M-CSF or IL-12/IL-18 and then further treated 7 d with each of these stimuli and simultaneously infected with HIV-1 BaL. Viral replication was quantified by ELISA measurement of p24 Ag in the supernatant. Mean ± SD of one representative experiment is shown. At least three independent donors were tested. (B) Monocytes were differentiated to macrophages for 7 d with M-CSF and then treated for the indicated days with IL-12/IL-18, washed with PBS, and infected for 7 d with HIV-1 BaL. Percentage of p24 production was calculated compared with a control of M-CSF MDM infected for 7 d in the presence of M-CSF. Bars represent mean ± SD of one representative experiment. At least three independent donors were tested.

**Results**

**HIV replication is restricted in IL-12/IL-18 MDM**

Monocytes were differentiated into macrophages in the presence of M-CSF, IL-12/IL-18, GM-CSF, and IL-4 or TNF-α. As expected, infection of M-CSF MDM resulted in a strong HIV-1 p24 Ag production 7 d postinfection (Fig. 1A, filled bars). GM-CSF or TNF-α–derived MDM led to detectable p24 Ag production in the cell supernatant (Fig. 1A, filled bars). Conversely, IL-12/IL-18 MDM did not produce p24 Ag (Fig. 1A), as levels were comparable to samples in which replication had been completely inhibited with 1 μg/ml reverse transcriptase inhibitor AZT, representing the background of viral input (Fig. 1A, open bars). Moreover, virus replication was potently inhibited as measured by proviral DNA determination (Fig. 1B). Replication of an X4, macrophage-tropic HIV-1 virus, 92UG024, was also severely restricted in IL-12/IL-18 when compared with M-CSF MDM (Fig. 1C), indicating that inhibition was not dependent on virus coreceptor use. Importantly, when monocytes were differentiated in the presence of M-CSF and then further treated with IL-12/IL-18, macrophages became resistant to HIV-1 infection (Fig. 2A), suggesting that IL-12/IL-18 triggered an antiviral restriction in macrophages. Conversely, treatment of IL-12/IL-18 MDM with M-CSF did not fully restore the capacity of HIV-1 to infect macrophages (Fig. 2A). To study how IL-12/IL-18 triggered this HIV-1 restriction, M-CSF–differentiated macrophages were treated for the days indicated with IL-12/IL-18, washed with PBS, and then infected with the HIV-1 BaL strain for 7 d. We observed that a 24-h treatment with IL-12/IL-18 was able to induce a restriction state in M-CSF macrophages, blocking the production of HIV-1 by >90% (Fig. 2B). Twenty-four hours infection of M-CSF MDM with HIV-1 BaL strain followed by IL-12/IL-18 or AZT treatment inhibited viral replication from subsequent infection cycles (Supplemental Fig. 1).

**HIV coreceptor expression was not significantly affected by IL-12/IL-18 treatment, and we failed to clearly quantify CD4 expression due to macrophage autofluorescence (Supplemental Fig. 2).** Nevertheless, IL-12/IL-18 MDM acutely infected with VSV-pseudotyped HIV-1 virus were also completely resistant to infection (Supplemental Fig. 3). Thus, these results indicate that the effect of IL-12/IL-18 was independent of virus entry but dependent on an intracellular event.

**SAMHD1 mRNA levels are increased in IL-12/IL-18 MDM**

We compared the mRNA levels and protein expression of SAMHD1 in M-CSF or IL-12/18 MDM. Relative levels of SAMHD1 mRNA were >10-fold higher in IL-12/18 MDM as compared with M-CSF MDM (Fig. 3A, filled bars). Conversely, mRNA expression of TRIM-5α, an alternative HIV restriction factor, was not significantly different between M-CSF and IL-12/IL-18 MDM (data not shown). The increased levels of SAMHD1 in IL-12/IL-18 MDM has a reflection at the protein level, as 7-d-differentiated IL-12/IL-18 MDM showed a stronger signal than did M-CSF MDM when immunoblotting for SAMHD1 (Fig. 3B).

**FIGURE 3.** SAMHD1 mRNA levels are increased in IL-12/IL-18 MDM. (A) Monocytes of two different donors were differentiated for 7 d with M-CSF, IL-12/IL-18, or IFN-γ in the absence (filled columns) or presence of 625 ng/ml of an anti-IFN-γ–neutralizing Ab. RNA was extracted and mRNA levels of SAMHD1 were quantified by real-time quantitative PCR. Levels of SAMHD1 mRNA were represented as fold induction relative to undifferentiated monocytes. Results corresponding to two of four donors are shown. (B) Immunoblot of monocytes differentiated for 7 d in the presence of M-CSF or IL-12/IL-18. The expression of a housekeeping gene (Hsp90) is used as control. Blots from four independent donors are shown. Densitometric analysis representing mean ± SD of these blots is shown on the right.
An anti-IFN-γ–neutralizing Ab did not have a significant effect on the upregulation of SAMHD1 by IL-12/IL-18 (Fig. 3A, open bars), suggesting that an autocrine, IFN-γ–dependent effect was not responsible for the upregulation of SAMHD1. Conversely, IFN-γ–induced upregulation of SAMHD1 was reduced in the presence of an anti-IFN-γ–neutralizing Ab (Fig. 3A, open bars).

Degradation of SAMHD1 restores infectivity of IL-12/IL-18

MDM

RNA interference was used to confirm the role of SAMHD1 in virus restriction imposed by IL-12/IL-18. As shown in Fig. 4, strong inhibition of SAMHD1 mRNA and protein levels were achieved after transfection of siRNA targeting SAMHD1 in M-CSF or IL-12/IL-18 MDM (Fig. 4A, 4B). Inhibition of SAMHD1 partially increased M-CSF MDM HIV-1 infection, measured as p24 Ag production 7 days postinfection, compared with mock-transfected macrophages or macrophages transfected with a nontargeting siRNA pool (Fig. 4C). As expected, RNA interference of HIV receptor CD4 also inhibited HIV-1 replication. siRNA-mediated inhibition of SAMHD1 allowed the replication of HIV-1 in IL-12/IL-18 MDM that could not be detected under any other siRNA treatment or in mock-transfected cells (Fig. 4C, open bars), suggesting that SAMHD1 was the main block for HIV-1 replication in IL-12/IL-18 MDM. Cell viability for all the conditions was measured by MTT assay and no toxic effect was detected upon any siRNA treatment (data not shown).

SAMHD1 is degraded by the lentiviral protein Vpx, expressed by HIV-2 or some SIV strains. We treated M-CSF and IL-12/IL-18 MDM with SIV VLP that deliver Vpx in the cytoplasm of the targeted cells, degrading SAMHD1 (Fig. 5A). Two hours after starting treatment, cells were infected with a VSV-pseudotyped, GFP-expressing, NL4-3 HIV-1 virus. Three days after infection, VLP treatment increased the percentage of infected M-CSF MDM from 2.5 to 8.7% (Fig. 5B). When we infected IL-12/IL-18 MDM, we found that the untreated cells were virtually resistant to the infection (Fig. 5C), but the treatment of the cells with VLP restores the infectivity, reaching 18.5% of GFP⁺ cells (Fig. 5C). M-CSF–treated cells are already susceptible to infection and the effect of Vpx may not be as profound as in cells in which HIV-1 cannot infect, explaining the lower levels of GFP⁺ cells reached by M-CSF MDM compared with IL-12/IL-18 MDM when both are treated with VLPs. We conclude that degradation of SAMHD1 by two different mechanisms restored HIV-1 infectivity in IL-12/IL-18 MDM, strongly indicating that SAMHD1 is the major restriction factor behind the IL-12/IL-18–induced MDM resistance to HIV-1 infection.

FIGURE 4. RNA interference of SAMHD1 eliminated HIV-1 restriction in IL-12/IL-18 MDM. (A) Monocytes were left untreated (mock-transfected) or transfected with 50 pmol of a nontargeting siRNA (siNT) or siRNAs targeting CD4 or SAMHD1 and then differentiated for 3 d with M-CSF (filled bars) or IL-12/IL-18 (open bars). RNA was extracted and relative levels of SAMHD1 mRNA were quantified by quantitative PCR and normalized to β2-microglobulin expression. One representative of three independent experiments is shown. (B) As in (A), protein was extracted and subjected to SDS-PAGE followed by immunoblotting using SAMHD1 and Hsp90 Abs. One representative blot is shown. (C) As in (A), transfected MDM were infected with HIV-1 BaL for 7 d. HIV-1 replication was quantified as p24 Ag production in M-CSF (filled bars) or IL-12/IL-18 MDM (open bars). Data represent the mean ± SD of one representative experiment. Three independent experiments were performed.

FIGURE 5. SAMHD1 degradation by SIV Vpx relieves infectivity restriction in IL-12/IL-18 MDM. (A) M-CSF and IL-12/IL-18–differentiated macrophages were left untreated or treated for 24 and 48 h with SIV VLP. Cells were lysed and SAMHD1 and Hsp90 levels were assessed by Western blotting. One representative blot is shown. (B) M-CSF– and (C) IL-12/IL-18–differentiated macrophages were left untreated (left panels) or treated for 2 h (right panels) with SIV-like particles carrying Vpx and then left uninfected (top panels) or infected (bottom panels) with a VSV-pseudotyped HIV-1 NL4-3 virus expressing GFP (NL4-3-GFP). Three days after infection, the percentage of GFP⁺ cells was measured by flow cytometry. One of two independent experiments is shown.
Discussion

Monocytes and macrophages have different susceptibility to infection by HIV depending on their origin, stage of differentiation, and environment. In this study we have shown that whereas human primary macrophage differentiated with M-CSF, GM-CSF/IL-4, or TNF-α become susceptible to HIV infection, stimulation of macrophages with a combination of IL-12 and IL-18 prevented or blocked a productive infection by HIV-1. Furthermore, 24 h stimulation with IL-12 and IL-18 was sufficient to make the cells resistant to HIV-1 infection.

The fact that macrophages can switch from a permissive to a nonpermissive state suggested that IL-12/IL-18 was inducing the expression or activation of a restriction factor. When we analyzed expression levels of SAMHD1, we found that mRNA and protein levels were increased in IL-12/IL-18 MDM (Fig. 3). We cannot discount that other factors may also be upregulated in IL-12/IL-18 MDM, especially when taking into account that we have previously shown that IL-12/IL-18 stimulation induced IFN-γ secretion that, in turn, could stimulate antiviral genes in an autocrine manner (6). Moreover, protein expression as measured by Western blot did not necessarily reflect the increased expression measured by mRNA quantification, suggesting that activation/deactivation of already expressed SAMHD1 could also be a mechanism affected by IL-12/IL-18. However, we have clearly demonstrated that the main HIV-1 restriction in IL-12/IL-18 MDM is due to SAMHD1, as RNA interference or Vpx-mediated degradation of SAMHD1 completely reverted the restriction in the IL-12/IL-18 (Figs. 4, 5).

The role of SAMHD1 in restricting HIV-1 replication in myeloid cells and lymphocytes has gained wide interest, but little is known about its regulation mechanism. SAMHD1 has been identified as an IFN-γ–dependent gene (25) (Fig. 3A), and IL-12/IL-18 macrophages produce significant amounts of IFN-γ (6). Then, we investigated whether SAMHD1 increased mRNA levels were induced by the IL-12/IL-18–stimulated production of IFN-γ acting in an autocrine manner. However, we have found that IL-12/IL-18 stimulation may increase SAMHD1 mRNA and protein levels independent of IFN-γ (Fig. 3A). To our knowledge, this is the first demonstration that SAMHD1 function can be modulated through an IFN-γ–independent pathway but, nevertheless, dependent on cytokines associated to the M1/Th1 differentiation process (4, 26). IL-12 and IL-18 are produced by APCs in response to the recognition of a pathogen. Furthermore, neutrophils produce very high levels of IL-12, and IL-18 is also produced by epithelial and endothelial cells, indicating that they might have a role in tissues rich in macrophage lung and mucosal tissues. IL-12 and IL-18 receptors are expressed in monocytes, macrophages, and lymphocytes (27–29). Production of IL-12 is thought to link innate immune responses with adaptive responses, leading to Th1 polarization of T lymphocytes. Therefore, the role of these and other cytokines in T cell virus restriction remains to be explored. SAMHD1 has been also described as a restriction factor in resting CD4+ T lymphocytes, which is lost when lymphocytes are activated (30, 31). Strikingly, no changes in SAMHD1 expression levels have been observed between resting and activated CD4+ lymphocytes (Refs. 30, 31, and E. Pauls and J. Este, unpublished observations), implying that additional regulation mechanisms may modulate SAMHD1 function. Therefore, we cannot exclude that IL-12/IL-18 may be activating some other mechanism of control of SAMHD1.

Finally, we have previously observed that IL-12 and IL-18 receptors are upregulated in CD4+ T resting lymphocytes of HIV-1–infected patients, suggesting that deregulation of the IL-12 and IL-18 pathways may play a role in the immunopathogenesis of HIV-1 infection (19). It is tempting to think that IL-12 and IL-18 signaling may link cell polarization to HIV-1 susceptibility through the regulation of SAMHD1. The observation that mutations in SAMHD1 are associated with the Aicardi-Goutières syndrome, a autoimmune illness characterized by elevated IFN levels (32), suggests that SAMHD1 may play a role in controlling or limiting the innate immune response to an unknown agent. SAMHD1 capacity to limit the pool of dNTPs may lead to abortive reverse transcription and the accumulation of intracellular DNA, which in turn may trigger an innate cellular response leading to apoptosis (33). How IL-12 and IL-18 may modulate this response through the regulation of SAMHD1 should be addressed in the future.

Acknowledgments

We thank the National Institutes of Health (AIDS Research and Reference Reagent Program) and the European Union European Vaccine Against AIDS Programme Centralised Facility for AIDS Reagents, U.K. National Institute for Biological Standards and Control, for reagents.

Disclosures

The authors have no financial conflicts of interest.

References


